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purpose: To ligate the purified vector + insert and transform with appropriate controls.

Ligation Rx:

Tested

Vector ~ 100 ng/μl	=	1 μl	1	Vector alone
Insert ~ 25 ng/μl	=	4 μl	1	" + real w/o
5x buffer	=	4	2	any purified
Ligase T4	=	1	3	Tag 1.5
H ₂ O	=	10	4	" 2
		20 μl	at 25°, 3 hr.	5 " + DV 1.5
				6 " " 2

transformation using DH5α Max eff. cells.

total volume used 2.5 μl of ligation Mix / transformation 50 μl cells

final volume after adding SOC ~ 500 μl

plated 25, 50 & 100 μl of each

Control diluted to 1:10 and plated 25, 50 & 100 μl.

normal transformation efficiency

Vector only - few blues because of the contamination ~ 1.5×10^9 no whites.

Vector + insert - ligated w/o any purification, lots + lots of colonies transformation quite efficient.

Tag + D.V } 2 mM Mg - didn't work

Tag alone } 1.5 mM Mg

Tag " } Very few colonies in 25 μl + 50 μl
Tag + D.V } 1.5 mM Mg no μl slightly better. No deep blue for better than Tag alone, however, are so few to make a call

- all purified gave low efficiency of transformation compared to un. " - vector + insert

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12/13/94

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